Isolation, characterisation and transferability of microsatellites for *Paraserianthes lophantha*, Cape Wattle (Leguminosae: Mimosoideae): a significant weed worldwide

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Introduction

Cape Wattle, *Paraserianthes lophantha* (Willd.) I.C.Nielsen (Leguminosae, Subfamily Mimosoideae, Tribe Ingeae), is a fast growing shrub/tree (to 15 m), endemic to south-west Western Australia (subsp. *lophantha*) and Indonesia (subsp. *montana* (Jungh.) I.C.Nielsen) (Cowan 1998; Nielsen *et al.* 1983). Subspecies *lophantha* was deliberately introduced to the east coast of Australia (Carr *et al.* 1992) and is now found throughout southern Australia, although there is a marked disjunction between populations of south-west Western Australia and eastern Australia. The exact limits of its natural geographic range are not clear as early collections (1848 – 1884) from several islands off Australia's south coast include subsp. *lophantha* (Cowan 1998; Robinson *et al.* 1981; Willis 1947).

Paraserianthes lophantha is an ecological, horticultural and economically important species, being planted for reforestation programs, as ornamental and shade trees, used as food, firewood, and also as substitute for soap (Nielsen *et al.* 1983, Nielsen 1992, Barneby and Grimes 1996, Lewis and Rico Arce 2005). The species has also become a significant weed worldwide. It is of such concern in South Africa researchers are investigating and implementing biological controls in an attempt to eradicate the weed (J. Wilson pers. comm.; Dennill and Donnelly 1991; Schmidt *et al.* 1999). Despite its ecological, horticultural and economic importance, nothing is known of the genetic variation within the species. Here we describe the first microsatellite markers specific to *P. lophantha*, which will be used to document the genetic structure and variation of the species throughout its current range to delimit the native distribution of this weedy species. The cross-transferability of these markers are assessed on genera of tribe Ingeae and species of *Acacia* s.s.

Abstract

We isolated microsatellite markers from *Paraserianthes lophantha* and screened these, plus eight loci from species of *Acacia s.s.* and *Pithecellobium*, for polymorphism in 42 individuals of *P. lophantha*: 20 from one native population and 22 from across the native and introduced range in southern Australia. Nine loci were polymorphic. Observed heterozygosity ranged from 0 to 0.800 (mean 0.389) and the number of alleles per locus ranged from 1 to 6 (average of 3.444) in the native population. Cross-species transferability of polymorphic loci was tested on eight species of *Acacia s.s.* and 11 of tribe Ingeae. Amplification success varied between loci and taxa; the ingioid taxa from South-East Asia and Australia amplified most successfully. These loci will be useful in understanding the genetic variation and control of this significant worldwide weed.

Keywords: Microsatellites; cross-species transferability; *Acacia sensu stricto*; tribe Ingeae.

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Materials and methods

Genomic DNA was isolated from 42 individuals of *P. lophantha* from dried leaves (herbarium specimens or silica gel dried) with the QIAGEN DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, California) as per the manufacturer's protocol (Appendix 1). Microsatellite DNA loci were isolated, from one individual, following the biotinylated enrichment procedure of Murphy *et al.* (2009), which is based on the methods of Gardner *et al.* (1999; 2008). Positive clones were directly screened for microsatellite inserts via the three primer approach of Gardner *et al.* (1999). Those products that produced a smear on the agarose gel indicated the colony most likely included a microsatellite motif, while a strong single band indicated that no microsatellite was present.

One hundred and fourteen microsatellite-containing sequences (98%) were identified through sequencing 116 positive clones. To identify and eliminate motifs with similar flanking regions, which may represent non-unique loci, all sequences were analysed in the program MicroFamily (Meglécz 2007). Fifty-four potentially unique microsatellite loci were identified. For the 28 loci with eight or more dinucleotide repeats and four or more trinucleotide repeats, and with suitable flanking region, primers were designed in Primer3 (Rozen and Skaletsky 2000), and checked in NetPrimer (http://www.premierbiosoft.com/netprimer/index.html). Primers with a GC content of approximately 50% and a low 3' complimentarity were chosen.

Initial screening of the 28 loci was conducted with unlabelled primers on three individuals, including the individual used in development, to test for amplification. Each 10 µl reaction contained 1 µl 10X buffer, 0.88 mM dNTPs, 0.5 U HotStar *Taq*, 1 µM forward primer, 1 µM reverse primer, 1–11 ng of DNA and H_2O. The following PCR programme was used: 95°C for 15 min followed by 30 cycles at 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec; and a final elongation step at 72°C for 10 min. PCR products were visualised on a 1.5% agarose gel stained with ethidium bromide. Fourteen loci (50%) were successfully amplified for 10 individuals from across the geographic range then screened on a polyacrylamide gel to test for polymorphisms: eight loci were polymorphic, one was monomorphic, one did not amplify, and alleles in four loci were unclear.

Primers developed for related taxa were also assessed for amplification in *P. lophantha*: nine loci from *Acacia mangium* Willd. (Butcher *et al.* 2000: Am30, Am136, Am164, Am018, Am173, Am387, Am465, Am502, Am770), 10 loci from *A. saligna* (Labill.) H.L.Wendl. (Millar and Byrne 2007: As2.04, As2.13, As2.17, As2.20, As2.34, As2.46, As2.47, As2.57, As2.61, As2.62) and six loci from *Pithecellobium elegans* Ducke (Chase *et al.* 1996; Dayanandan *et al.* 1997: Pel1, Pel2, Pel3, Pel5, Pel6, Pel7). Of these, five from *A. mangium* (Am136, Am30, Am465, Am502, Am770) and three loci from *A. saligna* (As2.17, As2.46, As2.47) amplified individuals of *P. lophantha*, however, no primers for loci from *Pi. elegans* amplified products.

Using the Schuelke (2000) method, fluorescently labelled PCR was performed on 42 individuals (Appendix 1) — 20 from one population in the native range (BD, Leeuwin Naturaliste National Park, Boranup Drive, WA) and one individual from each of 22 locations across the native and introduced distribution range in Australia — for 16 loci: eight polymorphic newly isolated *P. lophantha* loci and eight loci (Am30, Am136, Am465, Am502, Am770, As2.17, As2.46, As2.47) developed for species of *Acacia* (Table 1). Each 25 µl reaction contained 5 µl Hot5tar master mix, 0.15 µl of 10 µM forward primer with tail, 0.5 µl of 10 µM reverse primer, 0.2 µl of fluorescently labelled 'M13' primer, 1–11 ng of DNA and H_2O. The same PCR programme as the unlabelled PCR was used. The fragments were run on a 3730 DNA Analyzer (Applied Biosystems) with a G5500LIZ_3730 size standard. Allele size and analyses were done with PeakScanner version 1.0 (Applied Biosystems).

Results and discussion

Nine of the 16 loci were polymorphic in *P. lophantha* from across the range tested. Genetic diversity parameters and tests for Hardy–Weinberg equilibrium and linkage equilibrium for these nine polymorphic loci were performed with GENALEX v. 6.3 (Peakall and Smouse 2006; Table 1) and GENEPOP v. 3.3 (Raymond and Rousset 1995) respectively, with significance values adjusted for multiple tests using a sequential Bonferroni procedure (Hochberg 1988). Null allele frequencies were estimated using CERVUS (Kalinowski *et al.* 2007) in samples from a single population (BD) only. The observed and expected heterozygosity from the select population (BD) ranged from 0 to 0.800 and 0 to 0.753 respectively, with means of 0.389 and 0.454. The number of alleles per locus in BD ranged from 1 to 6, with an average of 3.44. When all individuals were included, the number of alleles per locus ranged

Table 1. Characterisation of polymorphic loci. Primer sequences, Gen8ank accession number, repeat motif, and diversity characteristics of six microsatellite loci from *Paraserianthes lophantha* and three loci from species of Acacia s.s. (As2.17 and As2.46 from Millar & 8yrne (2007); Am465 from 8utcher et al. (2000)). N indicates the sample size; Na indicates number of alleles; ND indicates not done; 8D indicates samples from one population; PIC indicates polymorphic information content; * indicates significance after corrections for multiple tests; HWE indicates Test for Hardy-Weinberg Equilibrium.

Locus		Primer sequence (5'-3')	GenBank accession no.	Repeat motif	N - all (BD)	Allele size range - all (BD)	Na - all (BD)	Ho - BD only	He - BD	PIC - BD only	Null allele freq - BD only	HWE *P* - BD only
New loci:												
Plop4	F:	AAACCAAGGTCTTCTCTGCTTC	HQ449177	$(GT)_{24}$	42 (20)	192-218 (197-218)	10 (6)	0.650	0.753	0.713	0.0698	0.015
	R:	ACTCCCTCTCTTTCCATCTCT										
Plop6	F:	TGAAATGAGGGAGACGAGGA	HQ449178	$(GT)_{8}$	39 (19)	122-128 (122-126)	4 (3)	0.105	0.194	0.185	0.4446	<0.0001*
	R:	CACACATGTTCTCTCCTTACCTTG										
Plop8	F:	TTTGGCAGTCAGCAGAAGG	HQ449179	$(GT)_{5}GC(GT)_{12}$	42 (20)	163-203 (187-203)	10 (5)	0.500	0.729	0.68	0.1803	0.255
	R:	TTCTTCAATTTCCGTTCCATC										
Plop11	F:	TGTCAAACACACCTATCCACA	HQ449180	$(CA)_{16}$	42 (20)	221-243 (229-241)	11 (6)	0.800	0.728	0.681	-0.047	0.933
	R:	GACCGTCGGATCTGGAAGT										
Plop12	F:	GCATGTGACAATGGATGATTTC	HQ449181	$(TC)_{6}$	41 (20)	223-227 (223)	2 (1)	0	0	0	ND	ND
	R:	CATTCCTTCGCCATTCATTC										
Plop18	F:	ATTGAAGCTGCCCTCACATT	HQ449182	$(GT)_{3}(GC)_{2}(GT)_{5}$	42 (20)	178-180 (178-180)	2 (2)	0.500	0.480	0.365	-0.0204	0.852
	R:	TGTTCGGCCTCTTCTTTCTC										
Existing loci:												
As2.17	F:	TCCTCGCTTCTCGACATTTT	EF194135	$(AC)_{7}(TC)_{7}$	35 (15)	132-150 (132-150)	4 (3)	0.133	0.480	0.412	0.5585	<0.0001*
	R:	GCTCGAACCTTTCAAACGAA										
As2.46	F:	GTTCTCTTGCCCTGTTTGCT	EF194138	$(TC)_{7}$	42 (20)	120-126 (120-122)	3 (2)	0.550	0.489	0.369	-0.059	0.575
	R:	AGGCTGGAAATAAATGGAGGA										
Am465	F:	TGGGTATCACTTCCACCATT	FJ667822	$(AC)_{23}$	36 (19)	148-201 (148-150)	11 (3)	0.263	0.234	0.216	-0.0643	0.933
	R	AGGCTGCTTCTTTGTGCAGG										

from 2 to 11, with an average of 6.33. In our test population (BD), two loci depart significantly from Hardy–Weinberg Equilibrium after adjustments for multiple tests: Plop 6 and As2.17. Null alleles estimates are high for these two loci indicating they are probably not useful for routine analysis. No locus combinations exhibited significant linkage disequilibrium.

For all loci, except Plop18, the total number of alleles found in the 22 representative individuals was greater than the number of alleles found in population BD (Table 1): Plop6, Plop12, As2.17 and As2.46 had one more allele; Plop4 had four additional alleles; Plop8 and Plop11 had five extra alleles; and Am465 had seven additional alleles. Locus Plop12 was invariable in population BD (homozygous for allele 223), although individuals from five of the introduced populations EP, KI, LE, PI, RC (Appendix 1) had allele 227 as well as allele 223

To assess cross-species transferability, eight species of *Acacia s.s.* and 13 species of tribe Ingeae from across the phylogeny (Brown *et al.* 2008; Murphy *et al.* 2010) were screened for the nine polymorphic loci using the same protocol as above. No samples amplified for four loci: Plop6, Plop8, Plop11 and Plop12 (Table 2). Of the other six loci, amplification success varied: Plop18 did not amplify in any species of *Acacia s.s.*, while those taxa of tribe Ingeae from South-East Asia and Australia were the most successful, where three to five loci amplified respectively (Table 2).

These markers will be used to document the genetic diversity in *P. lophantha* to investigate the natural distribution of *P. lophantha*, which will inform management of populations in eastern Australia. Understanding the genetic variation and phylogeographic history of southern Australian populations of subsp. *lophantha* will also improve our knowledge of the evolution of *Acacia s.s.*, as *P. lophantha*

Table 2. Cross-amplification of nine loci on 21 species related to *Paraserianthes lophantha*. Number of species amplified, allele size range and number of alleles are summarised for taxa of *Acacia* s.s. and tribe *Ingeae*, with allele data for individuals noted below. * indicates tribe *Ingeae* species endemic to South-East Asia and Australia. – indicates no amplification.

	Plop4	Plop6	Plop8	Plop11	Plop12	Plop18	As2.17	As2.46	Am465
***Acacia s.s.* (8 species)**									
Number of species amplified	2	0	0	0	0	0	4	2	6
Allele size range (bp)	188-198	n/a	n/a	n/a	n/a	n/a	138-142	120-122	138-214
Number of alleles (Na)	3	n/a	n/a	n/a	n/a	n/a	3	2	9
Ingeae (13 species)									
Number of species amplified	5	0	0	0	0	6	6	6	11
Allele size range (bp)	176-224	n/a	n/a	n/a	n/a	158-178	138-142	120-134	164-224
Number of alleles (Na)	6	n/a	n/a	n/a	n/a	5	2	6	13
Alleles by taxon									
Acacia langifalia	–	–	–	–	–	–	–	122	–
Acacia melanaxylan	–	–	–	–	–	–	142	–	192, 212
Acacia murrayana	188	–	–	–	–	–	–	–	146, 214
Acacia parvipinnula	–	–	–	–	–	–	138	–	142
Acacia penninervis	–	–	–	–	–	–	–	–	140, 146
Acacia pulchella	–	–	–	–	–	–	–	–	178
Acacia saligna	196,198	–	–	–	–	–	138, 140	–	138, 146
Acacia victoriae	–	–	–	–	–	–	142	120	–
Abarema jupunba	–	–	–	–	–	–	–	–	180, 192
Albizia lebbeck	194, 196	–	–	–	–	178	142	126	166, 204
*Archidendran kanisii**	–	–	–	–	–	158, 172	142	–	172, 176
*Archidendrapsis basaltica**	196,224	–	–	–	–	174, 178	–	–	178, 184
Balizia elegans	–	–	–	–	–	–	138	–	164
Calliandra falialasa	–	–	–	–	–	–	–	–	–
Cajaba arbarea	–	–	–	–	–	–	–	–	–
Ebenapsis ebana	176	–	–	–	–	–	–	132	188, 224
Enteralabium cyclacarpum	–	–	–	–	–	–	–	132, 134	178
*Pararchidendron pruinasum**	190	–	–	–	–	176	142	122	121, 224
*Paraserianthes taana**	–	–	–	–	–	178	142	120	166
Samanea saman	–	–	–	–	–	–	–	–	188
*Wallaceadendran celebicum**	178	–	–	–	–	178	142	128	188, 198

has recently been identified as the closest relative of the Australian acacias (Brown *et al.* unpub.). These loci may also be useful in identifying source populations of introduced plants for better targeting biological controls.

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Appendix 1 Individuals used for DNA isolation and screening. Forty-two individuals that were isolated and screened for microsatellite loci developed for *P. lophantho* (this study) and other loci of related taxa (Butcher *et al.* 2000; Millar and Byrne 2007; Chase *et al.* 1996; Dayanandan *et al.* 1997) are listed. Locality details for populations the individual come from are given and each is assigned to be in either the native or introduced range.

Individual	Population location (population abbreviation)	Native/Introduced
GB207A	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207B	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207C	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207D	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207E	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207F	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207G	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207H	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207I	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207J	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207K	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207L	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207M	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207N	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207O	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207P	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207Q	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207R	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207S	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB207T	Leeuwin Naturaliste National Park, Boranup Drive, WA (BD)	Native
GB203B	Boat Harbour Rd, WA (BH)	Native
JW1_CA	Cape Arid National Park, WA (CA)	Native
GB204A	Donnelly River, WA (DR)	Native
JW3	Graphit Road, WA (Graphit)	Native
GB205A	Gingilup Swamps Native Reserve, WA (GS)	Native
JW2	South of Manjimup, WA (Manjimup)	Native
GB217A	Morangup Nature Reserve, WA (MR)	Native
JW6	Porongorup, WA (Poro)	Native
GB215A	Serpentine River, Serpentine Falls National Park, WA (SR)	Native
GB208A	Leeuwin Naturaliste National Park, Van Tripp Rd, WA (VT)	Native
P01	Recherche Islands, WA (RC)	Native
GB212C	Wellington Dam Rd, Wellington National Park, WA (WD)	Native
GB220A	Waterfall Gully, SA (WG)	Introduced
HO104070	Craggy Island, TAS (CI)	Introduced
HO444136	Devils Tower, TAS (DT)	Introduced
GB226A	White Flat Rd, Eyre Peninsula, SA (EP)	Introduced
GB224E	Kangaroo Island, SA (KI)	Introduced
GB229A	Lakes Entrance, VIC (LE)	Introduced
GB219A	Phillip Island, VIC (PI)	Introduced
GB22BB	Putney's Rd, near Mt Gambier, SA (PR)	Introduced
HO56079	Rodondo Island, TAS (RI)	Introduced
MJB1960B	Wye River, VIC (WR)	Introduced